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## Preimaginal feeding habits of *Isoperla carbonaria* Aubert, 1953 (Plecoptera, Perlodidae)

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### Abstract

Feeding habits of *Isoperla carbonaria* Aubert, 1953 nymphs have been investigated in the Rio Alpetto, an Alpine creek of North-Western Italy. This species is characterised by relatively small dimensions and rheophilous habits, inhabiting streams above 350 m a.s.l. in the Alps, Apennines and Sicily. In this study, we analysed gut contents of 72 nymphs of this species, with the aim to investigate feeding preferences and to assess the existence of size-related shifts in the trophic spectrum. Nymphs were collected from a single reach, whose benthic coenosis was also determined. We detected evident trophic preferences in the diet: few taxa constituted the greatest part of the ingested items, independently from their availability in the substratum. Moreover, we observed that largest nymphs fed on a broader number of taxa. These findings are discussed and compared with data obtained from other Systellognatha species.

**Key-words:** Stoneflies; predator; diet; Alps, gut contents.

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## Introduction

Large invertebrate predators play a main role in many aquatic systems, where they act as top-down control elements in the benthic community (Wipfli & Gregovich 2002). In Alpine, fast flowing lotic systems these predators are mainly represented by Plecoptera Systellognatha. In these environments, carnivorous stoneflies can have a great ecological importance: for example Allan (1983) reported that dry weight of insect preys consumed by stoneflies could be about half that consumed by trout in a Rocky Mountain stream. In this frame, Perlidae and Perlodidae represent the most interesting subjects of study.

*Isoperla carbonaria* Aubert, 1953 (Perlodidae) is a rheophilous species, present in some Italian mountainous systems, with a total length of approximately 10-13 mm (Consiglio 1980). In Western Alps *I. carbonaria* is localised but quite common (Fochetti 1995), and its presence in the high Po Valley was firstly reported by Ravizza Dematteis & Ravizza (1988). As a Perlodidae, it is supposed that this species ~~could~~ behaves as a predator, but up to now nothing is known about its trophic role. The aim of this study is to describe the diet of *I. carbonaria* in a lotic system of the Western Alps, investigating the existence of feeding preferences and size-related trophic changes.

## Methods

In June and July 2006, *I. carbonaria* nymphs were collected in a 100 m uniform riffle of the Rio Alpetto (44° 40' 20.87''N, 7° 10' 18.78'' E; 1600 m a.s.l.). In the sampling station, Rio Alpetto is a typical alpine lotic environment, characterized by high slope, coarse riverbed and fast flowing waters. Riparian vegetation is almost absent, except for some *Sorbus aucuparia* descriptor, *Alnus viridis* descriptor and *Fraxinus excelsior* descriptor. This lotic system shows a good environmental quality, corresponding to an environment without trace of human-induced alteration (first class in the Italian Extended Biotic Index, Ghetti 1997). Samplings

were realized with a kick-net sampler (mesh size of 250  $\mu\text{m}$ ), early in the morning, because Systellognatha are considered to be chiefly nocturnal feeders (Vaught & Stewart 1974). Moreover, in the same reach we assessed the presence and abundance of taxa of the benthic community by using a Surber net (20  $\times$  20 cm; mesh 250  $\mu\text{m}$ ). Samples were preserved in 90% ethanol. In the laboratory, all organisms were counted and identified to genus level, except for few groups (e.g.: some Oligochaeta and early instars of some Diptera and Trichoptera), which were identified to family level. *I. carbonaria* nymphs were measured (total length - 0.1 mm accuracy) and processed to assess food consumption by means of gut contents analysis. For some of the small specimens (< 10.0 mm total length), the contents of alimentary canal were analysed following the transparency method proposed by Bello and Cabrera (1999) and widely employed in stonefly feeding studies (Tierno de Figueroa and Sánchez-Ortega, 1999; Tierno de Figueroa et al., 2003; Cammarata et al., 2007) with slight variations: each nymph was introduced in a vial with Herwitgs' liquid for 48 hours at 25°C, and afterwards, cleared individuals were collocated on a slide glass with a cover glass on. For large sized nymphs (> 10.0 mm total length), we removed the gut dissecting the specimens. Then we extracted and analysed contents of the alimentary canal. As previously observed by the authors, there is not difference in the obtained results as consequence of the use of both methods. Identification of prey was based on sclerotized body parts, particularly head capsules, mouthparts and leg fragments. To investigate the existence of feeding preferences, gut contents were compared with the natural composition and abundance of the macroinvertebrate community in the riverbed using the trophic electivity index of Ivlev (1961):  $E = (r_i - p_i) / (r_i + p_i)$ , where  $r_i$  = the proportion of ingested species and  $p_i$  = the relative abundance in the benthic community. Feeding preferences were also quantified using another electivity index (McCormick, 1991):  $E^* = (W_i - 1 / N) / (W_i + 1 / N)$ , where  $W_i = (r_i / p_i) / \sum r_i / p_i$ .

In this formula,  $r_i$  = the proportion of ingested species,  $p_i$  = the relative abundance in the benthic community, and  $N$  = the number of food items. Both indexes range from  $-1.0$  to  $1.0$ . A value of  $-1.0$  means total avoidance,  $1.0$  indicates preference and  $0$  indicates indifference.

## Results

In total we analyzed the diet of 72 *I. carbonaria* nymphs (from a total of 75 studied nymphs) and we collected and identified 1367 benthic macroinvertebrates from the riverbed. Benthic communities were composed of typical orophilous and stenothermic taxa (Table I). Mean length of the studied *I. carbonaria* immature stages was  $11.0 \pm 1.69$  mm SD, with a minimum of 6.50 mm and a maximum of 13.5 mm. Analyzing gut contents, we noticed that 19.0 % of nymphs had completely empty guts. In 53 specimens we detected the presence of vegetal detritus, hyphae and algae. Furthermore, in 12 guts we discovered the presence of sand and inorganic detritus. Results of the application of electivity indexes are reported in Figs. 1 and 2. Considering all specimens collected, the most important prey group in the guts were Diptera Chironomidae: they constituted nearby the 59.5 % of total ingested items, but represented only the 5.9 % of the substratum community. Interestingly, Ephemeroptera were scarcely consumed: for example Heptageniidae represented 23.3% of the community and 0.6 % of the gut contents and Baetidae respectively 22.3 % and 3.1 % (move the statement to discussion). We also noticed an evident change in the diet of nymphs of different size. Dividing specimens in three dimensional classes (smallest: total length < 8.5 mm; medium sized: 8.5-10.0 mm; largest: > 10.0 mm) we noticed that largest ones fed on a broader number of taxa (ANOVA  $F_{2, 69} = 2.86$ ,  $P < 0.05$  - Figure 3).

## Discussion

Because of their importance as top predators in small lotic environments, studies on carnivorous Plecoptera feeding habits can represent an useful tool to investigate lotic food

webs, behavioural and ecological aspects of stream organism relationships, but up to now there are few studies related to Alpine species (e.g. Fenoglio & Bo 2004, Maiolini & Silveri, 2005, Fenoglio et al. 2007, Silveri et al. 2008). A first interesting result to discuss is the presence of evident trophic selection mechanisms in *I. carbonaria*: diet was dominated by Chironomidae, with indeterminate Trichoptera, Baetidae and Simuliidae comprising secondary dietary items. It is well known that predaceous stoneflies forage across the surface of the substratum seeking preys mainly using their antennae, and that they can use hydrodynamic cues to distinguish between prey (Peckarsky & Wilcox 1989). Nevertheless, some studies noticed different preferences for prey (according to their size, mobility, hardness...) among predaceous species, clearly showing that selection for particular prey taxa is a trait which varies among different species of stoneflies (Molles & Pietruszka 1987, Williams 1987, Dudgeon 2000). Particularly in Alpine environments, Chironomidae can be discriminated and actively hunted by *I. carbonaria*, constituting the preferred food item. Thus, Chironomidae are relatively abundant in the study area, they are not particularly fast for escaping and they are easy to ingest and assimilate (due to their handled size and thinner exoskeleton), making them an optimum prey for a predator such as *I. carbonaria*, as also happens for some other European *Isoperla* species as *I. acicularis* (Despax, 1936) and *I. moselyi* (Despax, 1936) (Berthélemy & Lahoud 1981) or *I. rivulorum* (Pictet, 1842) (Silveri et al. 2008) (statement too long).

This work also partially supports the hypothesis that vegetal detritus could represent an important component of Systellognatha food, as reported by other authors (Lucy et al. 1990). Interestingly, these results confirm what has been found in other two Perlodidae species, which trophic habits were recently investigated in the same Alpine area: *Dyctiogenus alpinus* (Fenoglio and Bo, 2004) and *D. fontium* (Fenoglio et al. 2007): that is?.

Finally, we detected an increase in the trophic spectrum of nymphs related with nymphal size: smaller individuals fed on fewer taxa, while greatest ones captured a wide range of

organisms. Dudgeon (2000) also found that large nymphs of a *Kamimuria* species (a tropical Perlidae) ate a wider range of prey, but, as pointed out by this author, this can be attributed to the effects of sample size (what about the other studies carried out in Alpine streams?). This is not the case in our study, in which the broader diet of larger individuals could be due to the enhance in the ability of consuming different preys, also characterised by larger size [as previously pointed for some species of stoneflies (Sheldon 1980)], and/or in the capacity of exploring more microhabitats in the riverbed. Nevertheless, experimental studies are needed for confirming anyone of these hypothesis.

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**Figure captions:**

Table 1: List of macroinvertebrates collected in the Rio Alpetto station. FFG: functional feeding groups.

Figure 1: Ivlev's electivity index

Figure 2: McCormick's electivity index

Figure 3: Number of prey taxa in the guts of the three age groups of *I. carbonaria* nymphs (mean  $\pm$  se).

TAXA		FFG *
<b>Plecoptera</b>		
Leuctridae	<i>Leuctra</i> sp.	Sh
Chloroperlidae	<i>Chloroperla susemicheli</i>	P
Nemouridae	<i>Nemoura</i> sp.	Sh
	<i>Protonemura</i> sp.	Sh
Perlodidae	<i>Isoperla</i> sp.	P
<b>Ephemeroptera</b>		
Heptageniidae	<i>Ecdyonurus</i> sp.	Sc
	<i>Electrogena</i> sp.	Sc
	<i>Rhithrogena</i> sp.	Sc
	<i>Epeorus alpicola</i>	Sc
Baetidae	<i>Baetis</i> sp.	Cg
<b>Trichoptera</b>		
Odontoceridae	<i>Odontocerum albicorne</i>	Sh
Sericostomatidae	<i>Sericostoma</i> sp.	Sh
Polycentropodidae		F
Limnephilidae		Sh
Rhyacophilidae	<i>Rhyacophila</i> sp.	P
	<i>Hyporhyacophila</i> sp.	P
Philopotamidae		F
<b>Diptera</b>		
Thaumaleidae	<i>Thaumalea</i> sp.	Sc
Ceratopogonidae		P
Dixidae		Cg
Chironomidae		Cg
Limoniidae		P
Tipulidae	<i>Prionocera</i> sp.	Sh
Simuliidae		F
Empididae		P
<b>Coleoptera</b>		
Hydraenidae		Sc
Elminthidae		Cg
<b>Oligochaeta</b>		
Lumbricidae	<i>Eiseniella tetraedra</i>	Cg
Lumbriculidae		Cg
Naididae		Cg
<b>Platyhelminthes</b>		
Planariidae	<i>Crenobia alpina</i>	P

\* (Sh = Shredders, Sc = Scrapers, Cg = Collectors-gatherers, F = Filterers, P = Predators)